Kinetics of CO_2 Evolution, Soil Microbial Biomass Carbon, and Mineral-Associated Organic Carbon of a Tropical Soil Applied with Organic Matter

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ABSTRACT

The organic carbon of <53 μm particle size is mineral-associated organic carbon (MAOC), a stable soil organic matter (SOM) pool described by the CENTURY SOM model.

In a 110-day laboratory incubation experiment, we studied the effect of fresh organic matter (FOM) application: 0 (control); 1.81 g leaf litter (LL) carbon kg^{-1}; and 2.12 g (chicken manure (CM) carbon kg-1 in the MAOC, carbon dioxide (CO_2) evolution, and soil microbial biomass carbon (SMBC) of the 0–5- and 5–20-cm layers of a soil from Bagabag, Nueva Vizcaya, Philippines (121°15'E, 16°35'N).

CO_2 evolution rate was significantly higher in the 0–5- than in the 5–20-cm layer and decreased with time, peaking 3 days after FOM application. CM application significantly increased CO_2 evolution rate and cumulative CO_2 evolution in both 0–5- and 5–20-cm layers. Cumulative CO_2 evolutions in the LL-applied and control soils were statistically comparable.

SMBC was significantly higher in 0–5- than in 5–20-cm layer. CM application increased SMBC significantly compared to control and LL treatments. SMBC was higher at time periods 13 and 70 days after incubation (DAI) regardless of FOM treatment, indicating the presence of microbial energy in said periods.

FOM application significantly improved MAOC, and in agreement with previous studies, but our findings provide evidence of stable C turnover from MAOC in the short-term, challenging the convention that only labile SOC is involved in microbial CO_2 evolution from soils.

INTRODUCTION

Soil organic carbon (SOC) is the largest pool within the terrestrial carbon cycle (Gerzabek *et al.,* 2001). It consists of a heterogeneous mixture of organic matter originating from plant, microbial and animal residues (Baldock and Skjemstad, 2000). In recent years, a variety of terrestrial ecosystem models have been developed to study the impacts of management and/or climate change on SOC turnover under different climates, topographies and management (Sherrod *et al.,* 2005). For example, the CENTURY model is a terrestrial SOC model which partitions SOC into three conceptual pools:

active, slow, and passive, which differ in turnover times (Parton *et al.,* 1988). From the literature, we summarized the relationships of the measurable fractions of these conceptual pools and their measurable fractions with the particle size fractions (Table 1). The mineral-associated organic carbon (MAOC) is the measurable fraction of the passive SOC pool (Sherrod *et al.,* 2005). The MAOC fraction can be measured by physically separating the <53 mm particle size fraction, which is the silt- and clay-sized fraction (Haile-Mariam *et al.,* 2008). The associated SOC of the combined silt and clay is the MAOC (Cambardella and Elliot, 1992).

The particle size fractions play different roles in stabilization of soil organic matter (SOM). The major part of the SOM is usually associated with the clay- and silt-sized fractions (Ohm *et al.,* 2007). Fine-textured

Table 1. Matrix table indicating relationships of conceptual SOM pools, their measurable fractions, and particle size fractions.

Description	Conceptual SOM pools*		
	Active	Slow	Passive
1. Turn-over time	hours to months[2,4]; 2- to 4-years[8]	Decadal[2]; 20- to 50-years[8]	centuries to millennia[2]; 800–2000 years8
2. Representative SOM Fraction**	SMBC (soil microbial biomass carbon)[2,9,5,6]	POMC (particulate organic matter carbon)[7,2]	MAOC (mineral-associated organic carbon)[7,2]
3. Description of the fraction[8]	active soil organic matter (SOM) consisting of live microbes and microbial products	protected fraction that is more resistant to decomposition	physically-protected or chemically resistant and has long turnover time
4. Chemical composition[3]	chloroform-labile, microwave-irradiation-labile SOM, amino compounds, phospholipids	amino compounds; glycoproteins; aggregate protected POM; acid/base hydrolyzable; mobile humic acids	aliphatic macromolecules; charcoal; sporopolleins; lignins; high molecular, condensed SOM, humin, nonhydrolyzable SOM, fine silt, coarse-clay associated SOM
5. SOM fraction association with soil particle sizes	Fumigated and extracted SMBC[2,10]	2mm–53μm[2,7,1]; Sand-sized or larger[3]	<53 μm[2,7,1,] silt and clay-sized[1,2]*** referred to as MAOC in this paper

* The term "pool" is used to refer to the theoretically separated, kinetically delineated components of SOM
** The term "fraction" is used to describe measurable organic matter components associated with the pool
*** Silt and clay-sized particles were <53 μm diameter based on the USDA Soil Texture Classification System

[1] Haile-Mariam *et al.,* 2008
[2] Sherrod *et al.,* 2005
[3] Wander, 2004
[4] Follet, 2001
[5] Franzluebbers *et al*., 2000
[6] Franzluebbers *et al.,* 1996
[7] Cambardella and Elliot, 1992
[8] Parton, 1988
[9] Davidson *et al.,* 1987
[10] Vance *et al.,* 1987

soils have higher organic C and N contents than coarse-textured soils when supplied with similar input of organic material (Hassink, 1997). It was assumed that the difference was due to the ability of fine-textured soils to provide greater protection to soil organic matter (Hassink, 1997; van Veen and Kuikman, 1990), and physical protection of SOM is due to its ability to associate with clay and silt particles (Li *et al.,* 2007; Zhao *et al.,* 2005). The SOM associated with silt- and clay-sized fractions is often older than in the sand fractions, which is attributed to the stabilization mechanisms through surface interactions (von Lützow *et al.,* 2006; Rumpel *et al.,* 2004; Baldock and Skjemstad, 2000). Haile-Mariam *et al.* (2007) stated that the silt- and clay-associated C was older in the light fraction (LF) and particulate organic matter (POM) in all their study soils. Further, they disclosed that the clay-associated residues have the highest mean residence times (MRT).

Most of the input of carbon to soil from different sources is subject to microbial attack, explaining the extra CO_2 mineralization soon after addition to soil. A part, however, are retained and stabilized into the soil over long period of time. Previously, it was suggested that this extra CO_2 originates from the labile SOC fraction. However, in more recent studies, Hamer and Marschner (2005) stated that it seems unlikely that only the labile pool is affected, since it cannot fully account for the extra CO_2 released. Kuzyakov and Bol (2006) suggested that extra CO_2 evolution can originate from the various pools of SOM.

Some studies have found that organic matter (OM) application does not increase SOC (Foereid *et al*., 2004; Fontaine *et al.,* 2004; 2003; Bell *et al.*, 2003; Campbell *et al.,* 1991). Others have reported gains in SOC after years of OM addition to soil (Gerzabek *et al.,* 2001, 1997; Dalenberg and Jager, 1989).

In our experiment, we separated the soil microbial biomass carbon as a measure of the labile fraction using a modification of the fumigation extraction technique (Vance *et al.,* 1987) and the mineral-associated organic carbon fraction as a measure of the stable soil organic carbon fraction of a tropical soil by aid of chemical dispersion and physical fractionation (Haile-Mariam *et al.,* 2008; Sherrod *et al.,* 2005; Cambardella and Elliot, 1992.

Specifically, our objectives are to (1) determine the short–term influence of fresh organic matter (FOM) application on the dynamics of MAOC, a stable soil organic fraction, and (2) study the dynamics of SMBC and CO_2 evolution in soils applied with fresh organic matters. We hypothesized that although the MAOC is physically protected in the silt and clay fractions, it does contribute to C turnover in the short-term, although conventionally believed to be stable and turn over in centuries to millennial time scales.

MATERIALS AND METHODS

Sampling sites and soil collection

Soil samples were collected from the 0–5- and 5–20-cm layers of an upland field located in Bagabag, Nueva Vizcaya, Philippines (121°15'E 16°35'N). The Bagabag soil has alluvial parent material under tropical climate and has moderate amount of organic matter (Dagdag *et al.,* 1963). It is classified under the San Manuel soil series, which are fertile and very well-drained, and is considered to be one of the best soils for agriculture in the Philippines. The land use is citrus orchard and minimum tillage is practiced (Dumale and Perez, 2003). Some of the physico-chemical characteristics of the sample soil are presented in Table 2.

The soil samples were air-dried in the shade for 7 days, sieved through a 2-mm mesh screen, and stored at 4°C until use. Most of the plant residue was removed by flotation, followed by drying of the soil.

Fresh organic matter characteristics and preparation

Commercially-available processed fresh organic matters (FOM) leaf litter and chicken

Table 2. Some physico-chemical properties of the Bagabag soil, Nueva Vizcaya, Philippines.

Depth (cm)	Soil texture	Particle density (g cm^{-3})	Bulk density (g cm^{-3})	Organic C (g kg^{-1})			Total N (g kg^{-1})	C/N ratio
				TOC	MAOC	Labile SOC*		
0–5	sandy	2.56	1.18	20.72	11.53	9.19	4.88	4.24
5–20	loam[1]	2.56	1.27	10.9	10.02	0.88	3.74	2.91

* TOC less MAOC
[1] Dagdag *et al., (*1963)

Figure 1. Figure 1. The experimental unit. Acrylic tubing fitted with a septum mounted on cable grand served as the gas sampling port (A); Viewed from the bottom of the lid are the gas sampling port, inlet and outlet "cock-rubber stopper" assembly (B); the triangular boring in the bottle lid (C); the "cock-rubber stopper" assembly (D); and the assembled experimental unit (E).

manure procured in Tokyo, Japan were used in the experiment. The FOM were air-dried indoors for 7 days, pulverized, finely ground, passed through a 0.5-mm mesh screen and placed in sealed plastic bags and stored at 4°C until use. Prior to soil incorporation, the FOM were analyzed for total organic carbon and nitrogen contents. Leaf litter contains 362.7 g kg^{-1} C; 18.0 g kg^{-1} N; 20.1 C/N ratio and chicken manure has 424.9 g kg^{-1} C; 52.5 g kg^{-1} N; 8.1 C/N ratio, both on a dry weight basis. Moisture contents at incorporation were 15.6 and 14.2 %, respectively.

Incubation experiment

Transparent 500-mL glass bottles with plastic lid were used for incubation. One bottle represented one experimental unit (Figure 1).

Three holes, one 12.5-mm diameter and two 10-mm diameter, were bored on the bottle lid in a triangular fashion. A "cock-rubber stopper" assembly, which was inserted into the 10-mm holes made in the bottle lid, simultaneously served as air outlet of "old air" inside the bottles and air inlet of "new moist air" after every gas sampling day. This "cock-rubber stopper" assembly was made by inserting a three-way plastic cock (Top Corp., Japan) into a 14 x 15.5 x 10.5 mm rubber stopper.

Also, a self-designed 35-mm length acrylic tubing sealed with a rubber septum was fitted in the 12.5-mm diameter hole in the bottle lid through a cable grand. This tubing served as the gas sampling port for CO_2 evolution measurement. During assembly of the incubation bottle, a rubber gasket was also fixed in the bottle rim prior to sealing of the incubation bottles. All assembled incubation bottles were tested leak-free by immersing in a pail of water.

Each experimental unit consisted of 20-g soil samples adjusted to 50% of the soil's water-holding capacity. Incubation was conducted for 110 days at 20°C constant temperature. Prior to airtight sealing of each incubation bottle, FOM was evenly incorporated to the soil according to FOM treatment rates. Sufficient numbers of experimental units were prepared to allow for three replicates per treatment on each sampling day. Parameters were measured by destructive sampling at 3, 13, 21, 44, 70, 85, and 110 days after FOM application. For MAOC, measurement was also conducted at day zero. Experimental units totaled 126 for each soil.

Separation and measurement of the mineral-associated organic carbon fraction

This study used a combined chemical dispersion and particle size separation method based on the work of several authors (Haile-Mariam et al., 2008; Sherrod et al., 2005; Bell et al., 2003; Cambardella & Elliot, 1992) to separate the combined silt- and clay-sized fractions which contain the mineral-associated organic carbon (MAOC).

On each sampling day, 5-g subsample was placed in 100-mL plastic bottle and dispersed with 50 mL of sodium hexametaphosphate (5 g/L). The suspension was shaken in a reciprocating shaker (Yamato shaker model SA-31, Yamato Scientific Co., Ltd., Japan) overnight at 240 rpm. The soil suspensions were sieved with a 53-µm screen (Tokyo Screen Co. Ltd., Japan). During sieving, the particles retained in the screen were repeatedly rinsed with distilled water to ensure thorough separation of the <53 µm particle size fraction.

The resulting suspension was dried overnight at 70° C. This fraction contains the MAOC. The dried samples were finely ground manually using mortar and pestle and passed through an 80-µm sieve (Tokyo Screen Co. Ltd., Japan). MAOC was measured by dry combustion using a Sumigraph NC-90A NC analyzer (Sumika Inc., Japan).

Gas sampling and CO_2 evolution measurement

Gas samples for CO_2 evolution measurement were drawn from incubation bottles using a 10-mL plastic syringe (Nipro, Japan) fitted with 0.70 x 38.00 mm needle (Nipro, Japan). Transparent 7-mL capacity glass vials were used as sample containers. The sample vials were vacuumed to avoid atmospheric contamination. Prior to sampling, the vials were vacuumed by subjecting to 2 millibars suction for about 10 min. The vials were sealed with a rubber septum.

Prior to drawing gas samples, the air inside each incubation bottle was homogenized by alternate pumping and sucking using the sampling syringe 4–5 times. Seven mL of gas sample were drawn and injected into the sample vials. From there, 1 mL of gas sample was drawn and injected into a 16A Gas Chromatograph (Shimadzu Inc.). On each sampling day, after drawing gas samples, the air inside the bottles were flashed out and substituted with moist air through the inlet and outlet cocks mounted in the bottle lid. The inlet cock was connected to an air source passing through a tank of distilled water to moisten the air and maintain moisture inside the incubation bottles. The outlet cock was simultaneously opened while moist air flowed through the inlet cock at 2.5 kgf cm^{-2} for 3 min to ensure flushing out of "old air" from the bottles.

Soil microbial biomass carbon fumigation, extraction, and measurement

The soil microbial biomass carbon (SMBC) fumigation and extraction techniques used were slightly modified from the fumigation-extraction method described by Vance *et al.* (1987). On each sampling day, 5-g subsamples were placed in small Petri dishes and placed

inside a glass desiccator containing 40 mL of ethanol-free chloroform ($CHCl_3$) in a small beaker. To enhance vapor production, the beaker of $CHCl_3$ was immersed in a cup of hot water. The desiccator was sealed and placed in the dark at 25°C for 24 h. After 24 h the beaker of $CHCl_3$ was removed, and the residual $CHCl_3$ vapor in the soil was removed by repeated evacuation using a vacuum pump connected to the desiccator.

For extraction, the samples were transferred to 100-mL plastic bottles, diluted with 50 mL of potassium sulfate (0.5 M K_2SO_4), and shaken in an oscillating shaker at 240 rpm. After 30 min, the suspension was filtered using Whatman No. 42 filter paper followed by membrane filtration using 0.2-µm Millex syringe-driven filter units. A separate set of unfumigated samples was also prepared for use as controls. The filtered samples were analyzed using a Total Organic Carbon Analyzer (Shimadzu TOC-VCSN, Shimadzu, Inc.). SMBC was calculated using the formula, SMBC = 2.64Ec, where Ec is the difference between the organic carbon extracted from the fumigated and non-fumigated samples (Vance *et al.,* 1987).

Statistical treatment of data

Data were subjected to statistical analysis following the split-split plot design to compare and determine any significant differences between and among treatment means. The analysis of variance (ANOVA) was conducted using the SAS software (SAS Institute). Comparisons of means were done using the least significant difference (LSD) or the Duncan's multiple range test (DMRT) where appropriate.

RESULTS AND DISCUSSION

CO_2 evolution rate in the soil

The addition of leaf litter and chicken manure dramatically increased CO_2 evolution rates in the soil and was highest during the 0–3 day period (Figure 2). The soils receiving

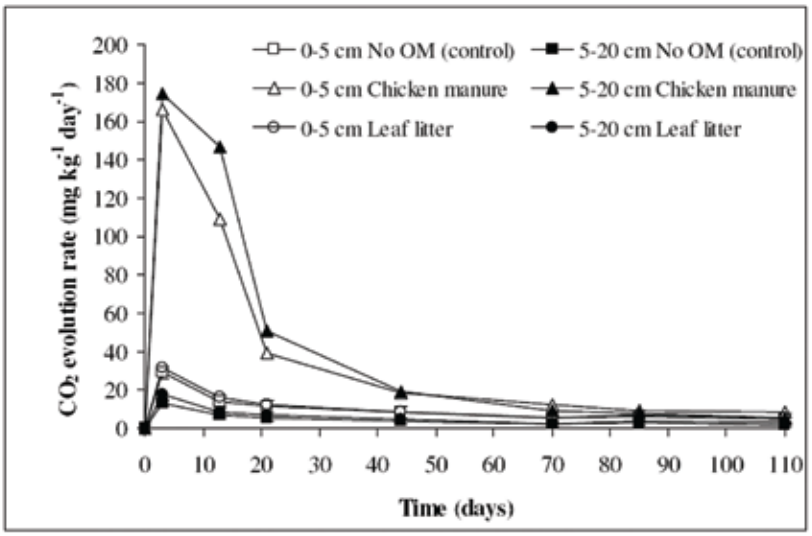

Figure 2. Carbon dioxide evolution rate (mg kg-1 day-1) of Bagabag soil, Nueva Vizcaya, Philippines over 110 days of incubation following application of leaf litter and chicken manure. Carbon dioxide evolution rate (mg kg-1 day-1) of Bagabag, Philippines soil over 110 days of incubation following application of leaf litter and chicken manure.

chicken manure showed exceptionally higher CO_2 production than those that received leaf litter both in the 0–5- and 5–20-cm layers. Increase in CO_2 evolution rates in soils applied with organic matters are reported by many authors. For example, Jacinthe *et al.* (2002) stated that daily CO_2 fluxes measured in late winter, summer, and autumn were generally higher in the wheat residue-treated plots than in the untreated plots.

Carbon dioxide is produced in the soil through the metabolism of plants roots, microflora, and fauna, and to a small extent, by chemical oxidation of carbon-bearing materials (Kuzyakov, 2006). The rate of soil CO_2 emission is normally controlled by several factors such as soil temperature, soil moisture and pore size (Raich and Schlensinger, 1992). It is also affected by agricultural practices such as tillage and residue management and varies with climatic conditions (Yavitt *et al.,* 1995; Osozawa and Hasegawa, 1995; Burton and Beauchamp, 1994; Fernandez *et al.,* 1993).

CO_2 evolution rate in the control and leaf litter-applied soils were 4.69 to 29.44 and 5.64 to 31.68 mg kg^{-1} day^{-1}, respectively in the 0–5-cm layer (Table 3). Statistical comparison of means showed that rates did not vary between all stages of the incubation period. In the chicken manure-applied soils, CO_2 evolution rates significantly varied, and highest during the 0–3-day period, following a decreasing trend through time. During the 0–3-day period, rate was 166.52 mg kg^{-1} day^{-1}. At 4–13 days after incubation, rate was 109.39 mg kg-1, which is significantly lower than the 0–3-day period. Further, CO_2 evolution rates during the period 14–110 days were comparable, and were significantly lower than rates during the 0–3- than the 4–13-day periods.

Also, in the 5–20-cm layer, CO_2 evolution rates in the control and leaf litter-applied soils both did not significantly vary in all stages of incubation. CO_2 evolution rates in the control soils ranged from 1.92 to 13.14 mg kg^{-1} day^{-1}. In the leaf litter applied soils, CO_2 evolution rate was in the range 2.96 to 17.83 mg kg^{-1} day^{-1}.

In the chicken manure-applied soils, CO_2 evolution rate during the 0– and 4–13-day periods were 174.92 and 146.57 mg kg^{-1} day^{-1}, respectively. These values were statistically comparable. Likewise, rates during the 14–21-, 22–44-, and 45–70-day

Table 3. CO_2 evolution rate in the 0–5- and 5–20-cm layers of Bagabag soil, Philippines as affected by fresh organic matter application and time (days after incubation).

	CO_2 evolution rate (mg $kg^{-1}day^{-1}$)					
	0–5-cm			5–20-cm		
Days after Incubation	No OM (control)	Leaf litter	Chicken manure	No OM (control)	Leaf litter	Chicken manure
0-3	29.44 a	31.68 a	166.52 a	13.14 a	17.83 a	174.92 a
4-13	13.56 a	15.9 a	109.39 b	6.64 a	8.67 a	146.57 ab
14-21	11.34 a	12.67 a	39.48 c	5.51 a	7.12 a	50.62 c
22-44	8.41 a	8.62 a	18.4 c	3.78 a	4.5 a	18.94 c
45-70	5.37 a	5.32 a	12.61 c	1.93 a	2.35 a	9.53 c
71-85	6.29 a	7.13 a	8.88 c	2.7 a	3.83 a	7.74 cd
86-110	4.69 a	5.64 a	8.8 c	1.92 a	2.96 a	5.03 d

In a column, means followed by different letters are significant at 5% level using DMRT

Table 4. Cumulative CO2 evolution in the 0–5- and 5–20-cm layers of Bagabag soil, Philippines applied with fresh organic matters.

FOM Treatment	Cumulative CO2 evolution (mg kg^{-1})	
	0–5-cm	5–20-cm
No OM (control)	906.82 b	396.96 b
Leaf litter (1.81 g kg^{-1})	1000.03 b	525.82 b
Chicken manure (2.12 g kg^{-1})	3100.38 a	3375.04 a

In a column, means followed by different letters are significant at 5 % by DMRT

periods were statistically the same, but all were significantly lower than in the 0–3- and 4–13-day evolution rates. CO_2 evolution rates further decreased significantly during the 71–110-day period, where CO_2 evolution rates were 5.03 to 7.74 mg kg^{-1}.

Calderon *et al.* (2004) observed that addition of manure increased CO_2 flux of the soils and that the largest difference between manured and control soils occurred at week 1, when the manured soils had from 42 to more than 400 % higher CO_2 fluxes. Similarly, Fontaine *et al.* (2004) confirmed that after a short lag phase (3 days) after cellulose addition, the cellulose decomposition followed an exponential dynamic until the rate of CO_2 production had markedly decreased (at day 17) likely due to cellulose exhaustion. Rudrappa *et al.* (2006) observed that the cumulative values of evolved CO_2-C increased rapidly from day 0 to 14, thereafter the increase was less for the rest of their incubation experiment. Clough and Kelliher (2005) added that maximum CO_2 production rate in the urine+dairy farm effluent-applied soils incubated at 28° C was attained starting immediately after application until day 5.

Cumulative CO_2 evolution

Fresh organic matter application induced higher cumulative CO_2 evolution compared to the control in both 0–5- and 5–20-cm layers (Table 4). Cumulative CO_2 evolution was 360 and 1060 % higher in the 0–5- and 5–20-cm layers, respectively compared to the control soils. This increase was significant as compared to both the control and leaf litter additions. The addition of leaf litter caused CO_2 evolution of 9.93 and 32.59% higher than the control in the 0–5- and 5–20-cm layers, respectively. These were not statistically higher than in the control soils. In the 0–5-cm layer, the CO_2 produced during the first 3 days after FOM application constituted 36.43 and 45.74% of the total CO_2 produced during the 110-day incubation of soils amended with leaf litter and chicken manure, respectively. In the 5–20-cm layer, CO_2 evolution constituted 37.74 and 42.32% of the total CO_2 evolved for soils treated with leaf litter and chicken manure, respectively.

The mean extra CO_2 evolution due to leaf litter application was 0.07 and 0.11 mg CO_2 kg^{-1} soil day^{-1} for the 0–5- and 5–20-cm layers, respectively. The application of chicken manure gave means of 2.59 and 3.43 mg CO_2 kg^{-1} soil day^{-1} in the 0–5- and 5–20-cm layers, respectively. Leaf litter application caused an extra CO_2 evolution of 93.21 (25.44 mg leaf litter C) and 128.86 (35.17 mg leaf litter C) mg kg^{-1}. This indicated that only 1.41 (0–5-cm) and 1.94 % (5–20-cm) of applied leaf litter carbon was mineralized in 110 days. In the chicken manure-applied soils, extra CO_2 evolution were 2193.56 (598.7 mg C) and 2978.08 (812.8 mg C) mg kg^{-1}, for the 0–5- and 5–20-cm depths, respectively, corresponding to extra C mineralization of 28.24 (0–5-cm) and 38.34% (5–20-cm) of applied chicken manure-carbon. These values indicate that the greater portion of applied FOM-C remained in soil after 110 incubation days.

It has been previously shown that added substrates are only partially mineralized. Of

the remainder, a fraction is immobilized by the microbial biomass as cell constituents and microbial metabolites. This fraction can be further metabolized and thus transformed to humified soil organic matter and CO_2. For example, Bremer and Kuikman (1994) added 30-µg glucose C g^{-1} soil and found an extra amount of CO_2-C evolved over the next 3 days equivalent to about 30% of the glucose-C added. Falchini *et al.,* (2003) found about 40% of added glucose mineralized after 7 days of incubation. Similarly, Jones and Shannon (1999) added about 30-µg C g−1 soil as an amino acids mixture to different soils and measured a mineralization rate of the substrate of the same order of magnitude (30–40%).

There are three categories of sources of CO_2 efflux from soils: (1) root respiration; (2) dissolution of inorganic carbonates; and (3) microbial respiration.

According to Kuzyakov (2006), there are four main contributors to CO_2 efflux classified as microbial: (1) microbial decomposition of soil organic matter in root free soil without undecomposed plant remains, frequently referred to as "basal respiration"; (2) microbial decomposition of soil organic matter in root affected or plant residue affected soil, called "rhizosphere priming effect" or "priming effect"; (3) microbial decomposition of dead plant remains; and (4) microbial decomposition of rhizodeposits from living roots, called "rhizomicrobial respiration". Root respiration and the dissolution of calcium carbonate ($CaCO_3$) also contribute to CO_2 efflux from soils. However, this $CaCO_3$ contribution during pedogenesis is only marginal since soil-CO2 flux measurements are usually done in sub-annual, annual, and decadal time scales. This indicates that CO_2 efflux from soils is largely controlled by the soil microbial population.

Soil microbial biomass carbon

Soil microbial biomass is the agent of mineralization of all of the plant and animal residues that enter soil. The degree of sequestration of soil organic carbon (C) is the result of the difference in the inputs to, and losses of, this C from the soil ecosystem, mediated by the microbial biomass. An understanding of the behavior of this biomass in the soil ecosystem is therefore essential if we are to understand the sequestration of C in soil. The microbial biomass uses two main carbon sources for energy: fresh inputs of plant and animal residues and humified soil organic matter. Fresh organic inputs of biological origin are most readily utilized and provide most energy per unit of carbon. Being largely polymeric, e.g., cellulose or protein, they can be efficiently degraded by the microbial biomass into their individual subunits of monosaccharide or amino acids etc. and assimilated into the microbial cells. During this process, typically 40 %–60 % of the organic C derived from the substrate is evolved as CO_2. Soil organic matter is basically formed from the action of the soil microbial biomass on these fresh inputs. Following repeated recycling by the biomass, they ultimately end up as forms of C which can be considered as biologically inert, having a half-life in soil of possibly thousands of years (Jenkinson *et al.,* 1992).

The soil microbial biomass increased dramatically and peaked 13 days after fresh organic matter application (Figure 3). The soils with chicken manure showed SMBC peaks of 1139.51 and 839.78 mg kg^{-1} in the 0–5- and 5–20-cm layers, respectively. In the soils receiving leaf litter, SMBC peaked at 666.86 and 469.48 mg kg^{-1} in the 0–5- and 5–20-cm layers, respectively. The 0–5-cm control peaked 699.25 mg kg^{-1} 13 days after incubation. On the other hand, in the 5–20-cm layer control, SMBC showed a decreasing pattern all throughout the duration of incubation, except when there was a slight

Table 5. Table 5. Soil microbial biomass carbon (SMBC) in the 0–5- and 5–20-cm layers of Bagabag soil, Philippines as affected by time (days after incubation) and fresh organic matter application.

	SMBC (mg kg^{-1})					
		0–5-cm			5–20-cm	
Days after Incubation	No OM (control)	Leaf litter	Chicken manure	No OM (control)	Leaf litter	Chicken manure
0-3	530.64 ab	456.98 bc	460.59 bc	424.16 a	450.82 a	178.99 b
4-13	699.25 a	666.86 a	1139.51 a	397.85 ab	469.48 a	839.78 a
14-21	250.54 c	216.48 d	308.26 c	376.73 abc	150.57 c	342.67 b
22-44	222.46 c	246.4 d	485.76 b	128.92 d	160.51 c	344.17 b
45-70	673.99 a	584.06 a	1059.52 a	357.98 abc	366.52 ab	693.79 a
71-85	390.98 b	296.91 cd	367.58 c	206.71 bc	470.45 a	316.45 b
86-110	454.17 b	538.56 ab	632.02 b	178.29 cd	175.82 bc	231 b

In a column, means followed by different letters are significant at 5% level using DMRT

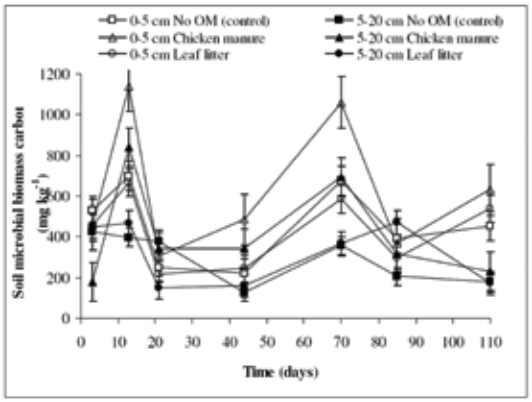

Figure 3. Changes in the soil microbial biomass carbon (SMBC) (mg kg-1) of Bagabag soil, Nueva Vizcaya, Philippines over 110 days of incubation following application of leaf litter and chicken manure.

increase at day 70. This was, however, lower than the day 3 level.

In the 0–5-cm layer, regardless of FOM treatment, SMBC was significantly and distinctly highest at two periods: 3–13-, and 45–70-day periods (Table 5). For the control soils, SMBC level 0–3 days after incubation was comparable with the 4–13-day level.

This pattern was also observed in the 5–20-cm layer. In the control soils, SMBC was significantly highest during the first 21 days of incubation and again at 45–70 days after incubation. In the leaf litter-applied soils, SMBC was highest during the first 13 days of incubation and again at 45–70 days after incubation. SMBC in the chicken manure treatment peaked 4–13-, and 45–70 days after incubation.

Several authors observed similar trend in terms of the surge in SMBC early in the incubation period. Lee *et al.* (2007) stated that annual application of manure caused a rapid increase in SMBC following application and potentially mineralizable C reached maximum fluxes within a month after manure application. De Nobili *et al.* (2001) pointed out that the microbial population is easily activated even by trace amounts of readily-available source of energy. Trace amounts of simple and easily degradable substances such as glucose or amino acids, and more complex soil and root extracts, could shift the soil microorganisms from dormancy to activity, causing more to be evolved as CO_2 than was contained in the substrate. This response of the microbial biomass is presumably in

anticipation of the coming of a bigger source of energy available for further reproduction and respiration. This could partly explain the response of SMBC almost immediately after FOM application. In conditions without any external application of readily-available substrates, favorable conditions of soil moisture or aeration would trigger this initial microbial response.

Increase in the SMBC (occurrence of the second peaks) was reported only in studies involving pulse additions of readily-available substrates (e.g. Hamer & Marschner, 2005).

Mineral-associated organic carbon

Original MAOC (day zero level) was slightly higher in the surface layer (0–5 cm) than in the 5–20-cm layer, with values of 11.53 and 10.02 g kg^{-1}, respectively (Table 2). In many cases, however, the surface layer of soils has less soil organic carbon content than the deeper layers primarily due to tillage, crop removal, and other practices (Blanco-Canqui and Lal, 2008; Anger & Eriksen-Hamel, 2008; Franzluebbers and Stuedemann, 2008; Potter *et al.*, 1998).

The kinetics of the mineral-associated organic carbon fraction in the 0–5- and 5–20-cm layers are shown in Figure 4.

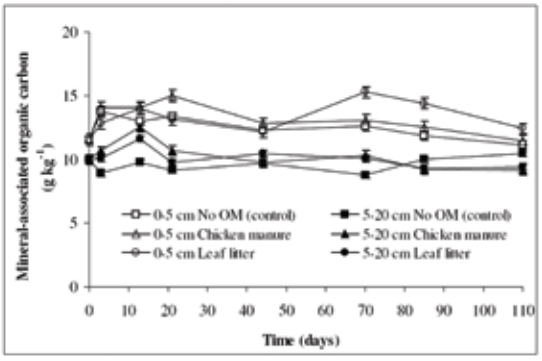

Figure 4. Mineral-associated organic carbon (MAOC) (g kg-1) of Bagabag soil, Nueva Vizcaya, Philippines over 110 days of incubation following application of leaf litter and chicken manure.

Between measurement dates during the incubation period, "add and subtract" changes in the MAOC particularly in the early stage of incubation were observed. These changes could have been due to the labile SOM that moves and associates with the particle size fractions. Haile-Mariam *et al.* (2008) disclosed that SOM is a continuum of materials from very young to very old with ongoing transfers between pools. This means that SOM moves between particle size fractions. Owing to artificial, biological, and other pedoturbations, the transfer of SOC between the particle size fractions is a continuous process in the soil continuum. However, it is assumed that the transfer of SOC from the silt- and clay sized fractions should be less than the transfer from the sand fractions to the finer-sized fractions, due to the physical protection of SOM by the silt and clay fractions (van Veen & Kuikman, 1990; Hassink, 1997). The organo-silt and organo-clay fractions in FOM are slow to mineralize due to physical protection (Mando *et al.,* 2005). This could result to the heterogeneity of SOC in the fine soil fractions because SOC from the sand-size fraction, from where SOM moves to the silt- and clay-sized fractions, is dominated by particulate plant material that has a lower extent of decomposition (Guggenberger *et al.,* 1995) and has younger

Table 6. Effect of fresh organic matter application on the mineral-associated organic carbon (MAOC) of Bagabag soil, Philippines.

FOM Treatment	MAOC (g kg^{-1})
No FOM (control)	10.96 b
Leaf litter	11.58 a
Chicken manure	11.59 a

Means followed by different letter(s) are significant at 5% level using DMRT

Table 7. Effect of time (days after incubation) on the mineral-associated organic carbon (MAOC) in the 0–5- and 5–20-cm layers of Bagabag soil, Philippines.

Days after Incubation	MAOC (g kg^{-1})	
	0–5-cm	5–20-cm
0	11.53 b	10.02 a
3	13.12 a	10.01 a
13	13.61 a	10.98 a
21	13.79 a	9.79 a
44	12.44 ab	9.89 a
70	13.59 a	9.79 a
85	12.69 a	9.54 b
110	11.52 b	9.68 ab

In a column, means followed by different letters are significant at 5% level using DMRT

radiocarbon ages (von Lűtzow *et al.,* 2006).

Leaf litter and chicken manure application significantly improved MAOC in the short-term (Table 6). This finding further confirms previous reports that organic matter application improves the soil organic carbon (Gerzabek *et al.,* 1997, 2001; Kogut, 1998; Mihaila and Hera, 1994; Dalenberg and Jager, 1989). Further, we separated statistically the effect of 110-day incubation on MAOC.

Incubation for 85 days resulted to a decrease in the mineral-associated organic carbon in the 5-20-cm layers (Table 7), from 10.02 to 9.54 g kg^{-1}. In the 0–5-cm layer, MAOC level at day zero and at 110 days after incubation were statistically the same, with values 11.53 and 11.52 g kg^{-1}, respectively.

We offer two possible explanations for the significant decrease of the mineral-associated organic carbon in short-term time scale. First, part of the MAOC could have been immediately used up by K-strategist microorganisms for respiration, leading to the immediate conversion to CO_2. Second, part of the MAOC could have been converted to more labile forms and used by the microbial biomass for cell division. This puts doubt on the strength of physical protection by fine soil fractions with time in soils receiving fresh organic matter.

Conclusions

The occurrence of second SMBC peaks in this experiment involving one-time only addition of fresh organic matters is very meaningful, and suggests a shift in the microbial community structure as the readily-available substrates from FOM became exhausted a few days after application. This suggests that the new soil microbial biomass growth found energy from a new source, which could be the stable SOM fraction. Regarding this process, Fontaine *et al.* (2003) suggested that most energetic compounds of FOM are used by r-strategist microorganisms that only decompose FOM. K-strategists arise only in the last stage of the FOM decomposition process when energy-rich compounds have been exhausted and only polymerized compounds remain.

Our finding of a significant MAOC decline in the 5–20-cm layer puts into question the convention that only the labile SOC contributes to CO_2 evolution in soils applied with FOM. This further suggests that physical protection of SOC in the silt and clay fractions is not a guarantee that it is resistant to turnover in the short-term time scale, although previously believed as such.

Findings of previous studies (Gerzabek *et al.,* 2001; 1997; Dalenberg and Jager, 1989) on SOC gains due to long-term C input were encouraging as these seem to prove the ability of soils to store carbon in the long-term. It is imperative, however, to elucidate the recalcitrance of the 'pierced' MAOC in the short-term. It is highly possible that this significant MAOC-derived C turnover came from the most recalcitrant and oldest SOM fraction. This could have big impact on the overall terrestrial carbon dynamics if the most stable SOC with long turnover times are lost in exchange of the less stable SOC that moves

into the fine soil fractions during carbon input to soil.

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